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Elso Lodi (*) & Valeria Marchionni (*)

THE KARYOTYPES OF *GAMBUSIA AFFINIS HOLBROOKI* GIR. FROM VIVERONE LAKE (ITALY) AND FROM AN ARTIFICIAL LAKE OF NICOSIA (CYPRUS)

(*Pisces Poeciliidae*)

Riassunto. — *Il cariotipo di* Gambusia affinis holbrooki *Gir. del Lago di Viverone (Italia) e di un lago artificiale di Nicosia (Cipro)* (Pisces Poeciliidae).

Negli ultimi cinquanta anni il cariotipo di *Gambusia affinis* è stato oggetto di studio da parte di numerosi ricercatori con risultati spesso contrastanti nelle diverse popolazioni, sia per quanto concerne il numero che per la morfologia dei cromosomi (2n = 36-48 cromosomi, presenza o assenza di un eterocromosoma metacentrico nel sesso femminile e di una coppia di cromosomi submetacentrici o acrocentrici). In due popolazioni di *G. affinis holbrooki*, una italiana ed una cipriota, si è potuto escludere l'esistenza di cromosomi eteromorfi e comunque si è appurata l'assenza del singolo cromosoma metacentrico nelle femmine.

Abstract. — The karyotype of *Gambusia affinis* has been investigated by several researches in the last fifty years and contrasting results have often been obtained in populations from different regions, both with regard to the number and to chromosome morphology (2n = 36-48 chromosomes, presence of absence of a metacentric heterochromosome in female sex and of a pair of acrocentric or submetacentric chromosomes). The existence of heteromorfic chromosomes has been excluded and the absence of the single metacentric chromosome has been ascertained in two populations of *G. affinis holbrooki*, one Italian and one Cyprian.

Introduction.

The mosquitofish are live-bearing, larvivorous, toothed carp. On account of this last outstanding feature they have been exported from North America into other continents in correlation with the biological fight against malaria, considering their striking ability to devour *Anopheles* larvae.

(*) Institute of Zoology of the University, Via Accademia Albertina 17, 10123 Torino (Italy).

Over the last fifty years several workers have focused their attention upon the sexuality and karyology from *Poeciliidae* of the *Gambusia* genus and particularly on the problem of the presence or absence of sex chromosomes in some species.

As early as 1924, attempting to explain the dearth of males recorded in *Gambusia affinis holbrooki* Gir., GEISER had inspected its spermatogenesis and, among other features, he had found a diploid complement of 36 and a haploid of 18 chromosomes. More recently, SHARMA and coworkers (1960, fide CHEN & EBELING, 1968) have obtained definitely different values (2n = 46) in Indian populations of *Gambusia affinis* (Baird & Girard), and another diverging finding was brought to notice by POST (1965), who reported a haploid complement of 24 chromosomes from as many as 79 teleostean species, including *Gambusia affinis holbrooki*. Subsequently, CHEN & EBELING (1968) on the basis of painstaking investigations on Californian and Texas populations of *G. affinis* have confirmed this last value (2n = 24) and have indicated as 48 the diploid chromosome complement in both sexes. Moreover, these last workers have brought to light the existence of female heterogamety in this species. In the male, 2 small submetacentrics, 44 acrocentrics of subequal length and 2 minute acrocentrics (ZZ chromosomes) were detected, whereas in the female one of the latter is replaced by a large metacentric (chromosome W). Preliminary observations in mosquitofishes from Florida have confirmed the presence of heterochromosome W. In the female complement (2n = 48) alone from *Gambusia gaigei* Hubbs, *G. hurtadoi* Hubbs & Springer and *G. nobilis* (Baird & Girard) CAMPOS & HUBBS (1971) also reported the presence of a metacentric chromosome (varying in length in different species), which on the contrary was lacking in *G. regani* Hubbs, *G. vittata* Hubbs (2n = 48) and in *G. marshi* Minckley & Craddock (2n = 42).

In a study on the evolution of the karyotype in *Poeciliidae* and *Cyprinodontidae,* SCHEEL (1972) has described a diploid complement made up of 48 acrocentrics in *Gambusia* species from the Canaries. Conversely, HINEGARDNER & ROSEN (1972) have indicated as 18 the haploid complement from *G. affinis holbrooki*. The female heterogamety in *G. affinis* detected by CHEN & EBELING (1968) was later confirmed by ITAHASHI et al. (1975, fide OJIMA et al., 1976) in Japanese specimens, in which however the presence of the two submetacentric autosomes was not recorded. Recently, CATAUDELLA & SOLA (1977) have not found any heteromorphic chromosome in *G. affinis holbrooki* specimens collected in several ponds near Rome, since their 48 chromosome complement was identical to that of *G. affinis* males studied by CHEN & EBELING.

From the literature quoted above it may be inferred that the karyological findigs, notably those related to *G. affinis*, though numerous, often differ between the various populations assayed. Accordingly, an attempt has been made by us to check the chromosome complement from two *G. affinis holbrooki* populations, one Italian and the other from Cyprus, in which sexuality is now being studied.

Materials and Methods.

Gambusia affinis holbrooki Gir. specimens collected in Viverone lake (Vercelli, Italy) and in an artificial pond near Nicosia (Cyprus) were used. Conceivably, the former population is a descendant from the strain introduced into Italy in 1922, derived from a stock acclimated in Spain, which in turn had been imported from North Carolina, where it had been collected near Edenton (DULZETTO, 1928). As to the other population (according to some information provided by the Fishery Department of the Ministry of Agriculture and Natural Resources of Nicosia), the mosquitofishes are derived from a strain imported from Siria in 1939 (Rockefeller project against malaria).

For the karyological investigation ml 0,01 of 0,1% colchicine solution was injected intraperitoneally to living individuals of *G. affinis holbrooki*. Kidneys, gills and gonads of the treated individuals were dissected out 4 hour after the colchicine injection and fixed in a solution containing 3 parts of methyl alcohol and 1 part of acetic acid after treatment in a hypotonic solution (0,35% sodium citrate) for 40 minutes. Preparations were made according to the routine air-drying methods (DENTON, 1973) and the staining was performed with Giemsa solution. The morphology of the chromosomes was designated according to LEVAN et al. (1964).

Results and Discussion.

Some 150 metaphase plates from 16 *Gambusia affinis holbrooki* specimens of both sexes from Viverone Lake, and the same number from the Cyprus basin, have been examined.

The diploid complement turned out to be similar in the two mosquitofish populations, and made up of 48 chromosomes. In addition, morphological differences in the karyograms between sexes were not evidenced (Figs. 1-8); in particular, in contrast to reports of CHEN & EBELING (1968) and ITAHASHI et al. (1975), we did not find any pair of heterochromosomes.

Fig. 1. — *Gambusia affinis holbrooki* ♀ (Viverone). Mitotic karyotype.

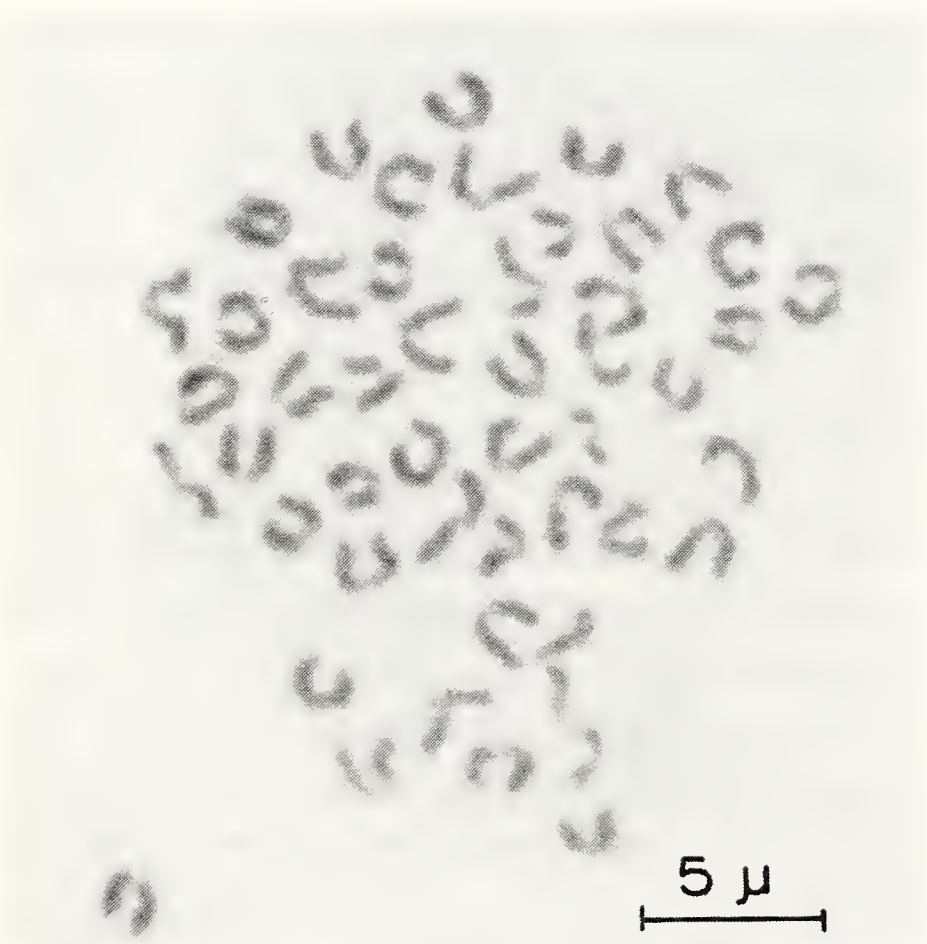

Fig. 2. — The same. Mitotic metaphase of a gill epithelial cell.

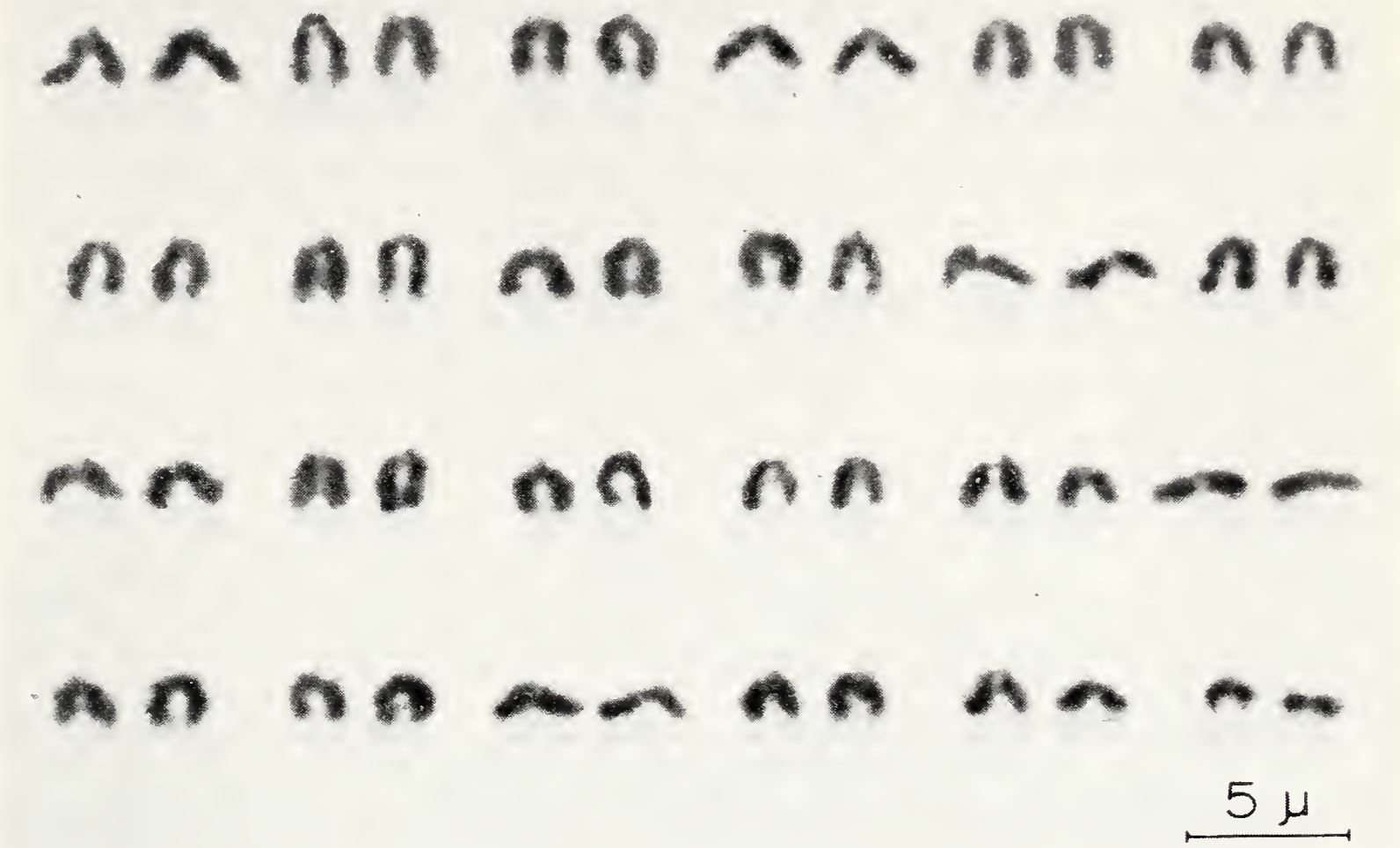

Fig. 3. — *Gambusia affinis holbrooki* ♂ (Viverone). Mitotic karyotype.

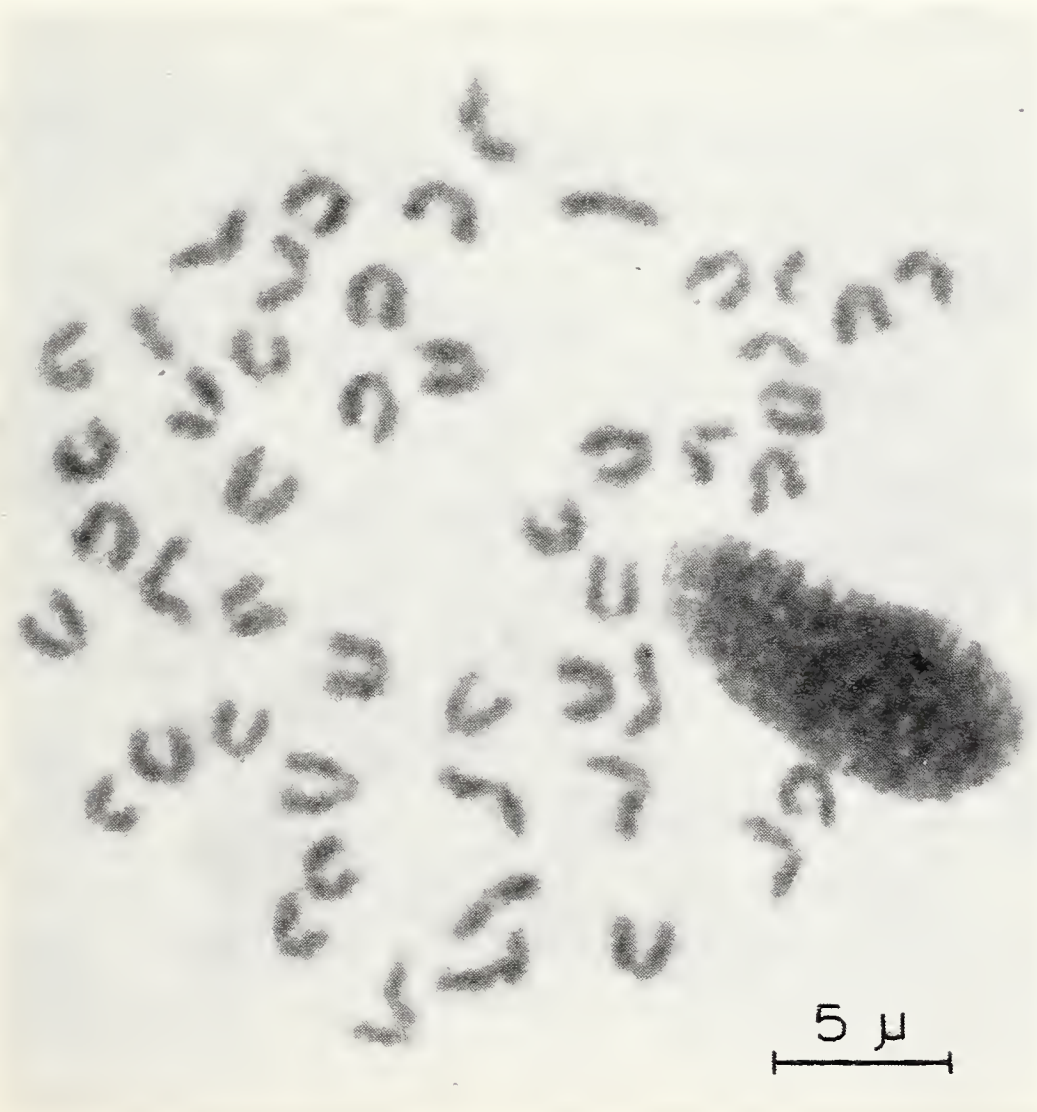

Fig. 4. — The same. Mitotic metaphase of a gill epithelial cell.

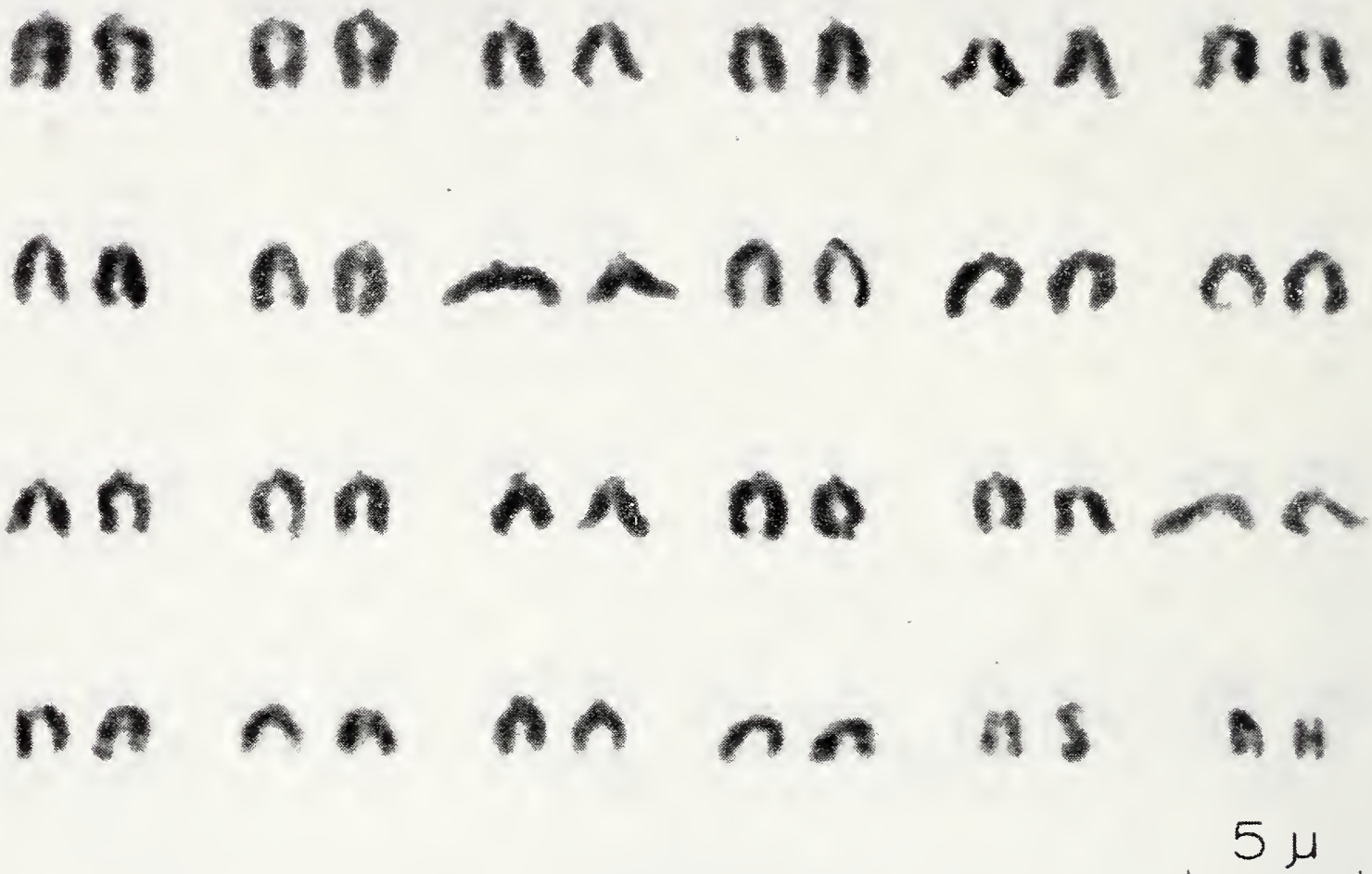

Fig. 5. — *Gambusia affinis holbrooki* ♀ (Cyprus). Mitotic karyotype.

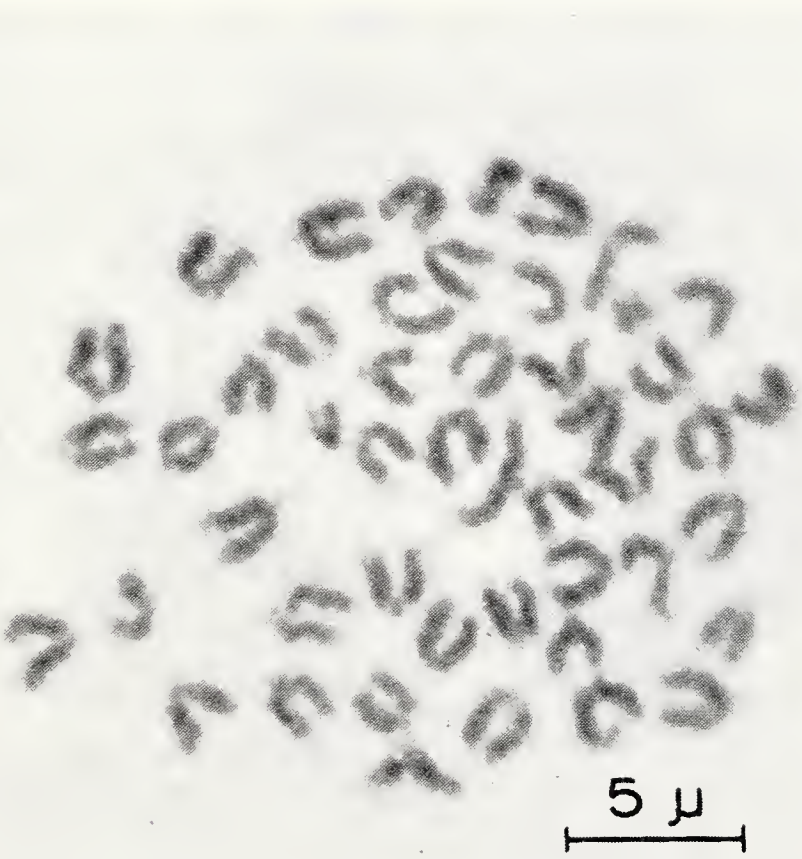

Fig. 6. — The same. Mitotic metaphase of a gill epithelial cell.

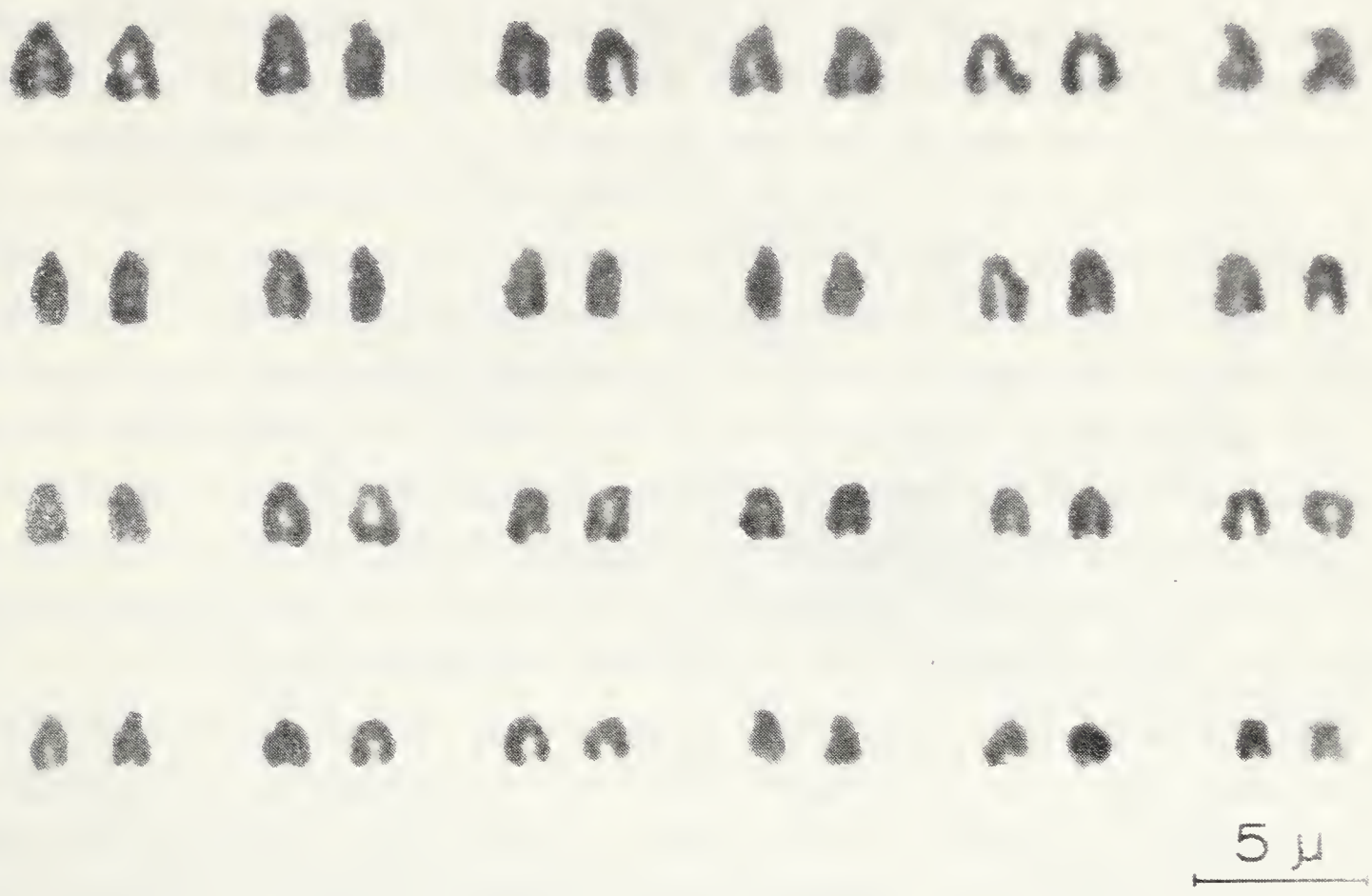

Fig. 7. — *Gambusia affinis holbrooki* ♂ (Cyprus). Mitotic karyotype.

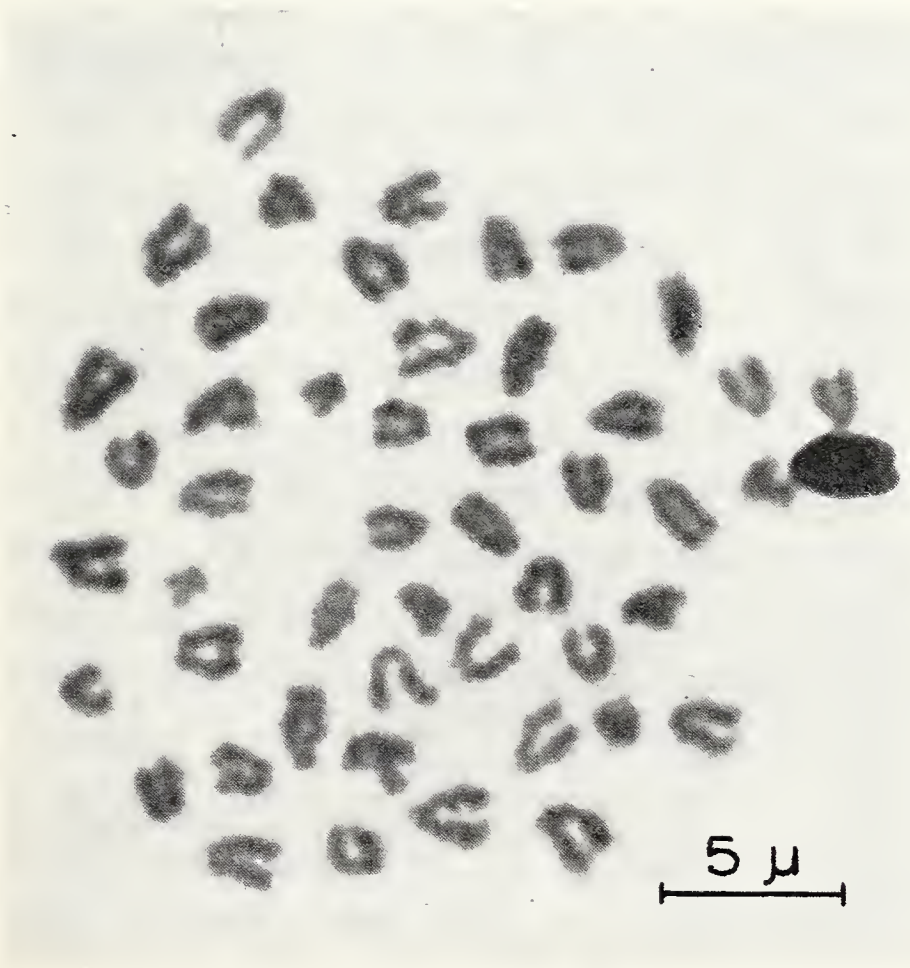

Fig. 8. — The same. Mitotic methaphase in testis cells.

In both *G. affinis holbrooki* strains assayed here, the karyotype consists of 24 chromosome pairs, approximately ranging in size from 1.3 to 3 μ. Chromosome length decreases gradually, thereby precluding their subdivision into groups according to size; moreover, in many instances homologous pairs are ill-defined, and hence a two-by-two chromosome arrangement must be viewed as a merely tentative pairing. As shown by the Figures, the centromere appears localized in the chromosome terminal region, therefore, after the nomenclature advised by LEVAN and coworkers (1964) they should be considered t chromosomes; as a consequence, the fundamental number is NF = 48. Nevertheless, it should be stressed that some doubt arises as to the classification of the smallest pair, its components appearing as a rule like t chromosomes, but seemingly exhibiting a centromere in their submedian region. Their minute size (below 1.5 μ) does not allow a clear resolution to be attained, thus explaining the fact that these chromosomes have been regarded as acrocentrics or submetacentrics by different investigators.

The chromosome complement (2n = 36) found by GEISER (1924) may be assumed to be erroneous, this being justified by the unsatisfactory techniques available at that time. Surprisingly, however, the above result has recently been substantiated by HINEGARDNER & ROSEN (1972) in mosquitofish of unknown derivation: rechecking these findings on the same material would therefore seem desirable.

As to the finding of 2n = 46 reported by SHARMA and coworkers (1960) by chromosome counts in Indian specimens, the interpretation forwarded by CHEN & EBELING (1968) may be quoted. According to these authors, Robertsonian chromosomal rearrangements being excluded; since both Indian and Californian populations are originally descended from the same dispersal centre in Mexico, the differences between them, if real, would have been due to rapid cytological differentiation affecting both the autosome number and the heterogametic mechanism.

Concerning the problem of the existence of cytologically identifiable sex chromosomes in *Gambusia affinis*, the data obtained in the present study on the subspecies widespread in Italy and Cyprus are the same as those reported by CATAUDELLA & SOLA (1977) from populations near Rome. This situation may therefore be claimed to occur most probably throughout the Italian and Cyprus island territories. In addition, present findings may be assumed to correspond to those of another Sirian population at least, from which the Cypriote mosquitofish derived after the 1939 importation.

In the light of the foregoing, further extensive investigations on the populations from the United States (in particular North Carolina, from which the Italian mosquitofish are derived) and from Mexico would be of interest in order to verify the two possible hypotheses on the origin of this chromosomal polymorphism. In fact it should be admitted that either (1) a different chromosomal rearrangement took place within a very short time interval in some populations imported to various continents, or (2) that a variability in the chromosomal complement already existed in the Mexican and United States populations of this species. Conceivably, the latter hypothesis is more verisimilar, in that it was shown by genetical studies that in the same poeciliid family *Xiphophorus maculatus* (Guenther) exhibits a clear polymorphism of its sex chromosomes (GORDON, 1954; KALLMAN, 1965), with some populations displaying female heterogamety, others male heterogamety and yet others with a mixed sexual type, in which sex determination falls within the WY - YY and XX - XY mechanisms.

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